

ENDOGENOUS RETROVIRAL SEQUENCES, ASSOCIATED WITH  
AUTOIMMUNE DISEASES AND/OR WITH PREGNANCY DISORDERS

Background of the Invention

B  
IMS D17  
The present invention relates to a new nucleic  
5 material of the endogenous retroviral genomic type,  
various nucleotide fragments comprising it or which are  
obtained from said material, as well as their use as  
marker for at least one autoimmune disease or a  
pathology which is associated with it, a pathological  
10 pregnancy or an unsuccessful pregnancy.

The screening of the cDNA library with the aid  
of the Ppol-MSRV probe (SEQ ID NO: 29) has made it  
possible to detect overlapping clones allowing the  
reconstruction of a putative genomic RNA of  
15 7582 nucleotides. - Reconstructed sequence is under-  
stood to mean the sequence deduced from the alignment  
of the overlapping clones -. This genomic RNA has the  
structure R-U5-gag-pol-env-U3-R. A "blastn" inter-  
rogation on several databases, with the aid of the  
20 reconstructed genome, shows that a large quantity of  
related genomic sequences (DNA) exist in the human  
genome. About 400 sequences have been identified in  
GenBank (cf Figure 3) and more than 200 sequences in  
the EST (Expressed Sequence Tag) library, the majority  
25 as antisense. These sequences are found on several  
chromosomes, in particular chromosomes 5, 7, 14, 16,  
21, 22, X, with a high apparent concentration of LTR on  
the X chromosome.

The reconstructed sequence (mRNA) is integrally  
30 contained inside the genomic clone RG083M05  
(gb AC00064) (9.6 kb), and exhibits 96% similarity with  
two discontinuous regions of this clone which also  
contains repeat regions at each end. The alignment of  
the experimental sequences corresponding to the 5' and  
35 3' regions of the reconstructed genomic RNA with the  
DNA of the RG083M05 clone has made it possible to  
deduce an LTR sequence and to identify elements  
characteristic of retroviruses, in particular those

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involved in reverse transcription, namely the PBS (Primer Binding Site) downstream of the 5' LTR and the PPT (PolyPurine Tract) upstream of the 3' LTR. It is observed that the U3 element is extremely short in comparison with the mammalian type C retroviruses, and comparable in size to the U3 region generally described in the type D retroviruses and the avian retroviruses. The PBS region is homologous to the PBS of the avian retroviruses, suggesting the use of the tRNA<sup>Trp</sup> as primer for the reverse transcription. Consequently, this new family of HERV is called HERV-W (Human Endogenous RetroVirus).

Phylogenetic analysis in the pol region has shown that the HERV-W family is phylogenetically linked to the ERV-9 and RTVL-H families, and therefore belongs to the family of type I endogenous retroviruses. Phylogenetic analysis of the open reading frame (ORF) of env shows that it is closer to the type D simian retroviruses and the avian reticuloendotheliosis retroviruses than type C mammalian retroviruses, suggesting a C/D chimeric genome structure.

The phylogenetic trees, supported by high "bootstrap" values show that the ERV-9 and HERV-W families are derived from two waves of independent insertions. Thus, the active element(s) at the origin of the HERV-W family is (are) different from that (those) from which the ERV-9 family is derived. Furthermore, the PBS of HERV-W probably uses a tRNA<sup>Trp</sup> whereas ERV-9 probably uses a tRNA<sup>Arg</sup>.

Finally, the members of the HERV-W family are expressed in the placenta, whereas the ERV-9 RNAs are not detected in this tissue.

#### BIOLOGICAL FUNCTIONS OF HERV-W

The expression of HERV-W restricted to the placenta and the long reading frame potentially encoding a retroviral envelope make it possible to propose physiological biological functions whose impairment could be associated with pathologies.

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5 The expression restricted to the placenta suggests that the expression of retroviral and/or nonretroviral genes under the control of the LTRs may be hormone-dependent. These genes may be adjacent, or under the control of isolated LTRs. A pathology may then result from an aberrant expression following the reactivation of a silent LTR by various factors: viral infection (for example by a member of the Herpesvirus family) or local immune activation. A polymorphism at  
10 the level of the LTRs could also promote these events.

15 The envelope of HERV-W could play a fusogenic role, in particular at the level of cellular subtypes of the placenta. An immunosuppressive peptide of this envelope could protect the fetus against attack by the maternal immune system. Finally, by a mechanism of saturation of receptors, the envelope of HERV-W could play a protective role against exogenous retroviral infections. The impairment of local cellular immunity may result from an immunostimulatory signal carried by  
20 the envelope. This effect may be linked to a region carrying a superantigen activity, or to the immunosuppressive region which would become immunostimulatory following either a polymorphism or a dose-effect (overexpression).

25 Verification of these implications and understanding of the consequences linked to an impairment of the biological functions of the endogenous LTRs or the retroviral envelope may lead to the establishment of methods of diagnosis or of monitoring:

30 - of states of pathological pregnancy or of unsuccessful pregnancy,

- of autoimmune diseases such as multiple sclerosis or rheumatoid arthritis.

*Summary of the Invention*

B In accordance with the present invention, there  
35 has been discovered, in the endogenous state, a new nucleic material, stated explicitly and described below, having the organization of a retrovirus, and capable of being correlated with an autoimmune disease,

or a pathology which is associated with it, with a pathological pregnancy or an unsuccessful pregnancy.

The nucleic material according to the present invention, in mRNA form, represents about 8 Kb; it is represented in Figure 1 and is described by SEQ ID NO: 11, and is represented in Figure 2 in the form of genomic DNA.

The expression "of retroviral type" is understood to mean the characteristic according to which the nucleic material considered comprises one or more nucleotide sequences related to the organization of a retrovirus, and/or to its functional or coding sequences.

This reference nucleic material is related to a human endogenous retrovirus, designated by the expression HERV-W. Consequently, it may be obtained by any appropriate technique for screening any library of human DNA, or of placental cDNA, as shown below, in particular with nucleic primers or probes synthesized so as to hybridize with all or part of SEQ ID NO: 11.

The present invention also relates to any nucleic or peptide product, obtained or derived from the reference nucleic material, according to SEQ ID NO: 11.

And finally, the invention relates to the various correlations which may be made between the abovementioned nucleic material, and/or its derived products, with any autoimmune disease and/or a pathology which is associated with it, as well as with cases of pathological pregnancy or of unsuccessful pregnancy.

"Autoimmune" is understood to mean in particular:

- multiple sclerosis
- rheumatoid arthritis
- disseminated lupus erythematosus
- insulin-dependent diabetes
- and/or pathologies which are associated with them.

The present invention relates, first of all, to a nucleic material of the retroviral genomic type, in isolated or purified state, at least partially functional or nonfunctional.

5 This material is characterized in that its genome comprises a reference nucleotide sequence chosen from the group including the sequences SEQ ID NOs: 1 to 15, their complementary sequences, and their equivalent sequences, in particular the nucleotide sequences  
10 exhibiting, for any sequence of 100 contiguous monomers, at least 50% and preferably at least 70%, for example at least 90% homology with respectively said sequences SEQ ID NOs: 1 to 15.

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15 This material is also characterized in that its genome comprises a reference nucleotide sequence, encoding any polypeptide exhibiting, for any contiguous sequence of at least 30 amino acids, at least 50%, and preferably at least 70% homology with a peptide sequence capable of being encoded by at least a  
20 functional part of the reference nucleotide sequence as defined above.

In particular, this material comprises a nucleic fragment inserted between two sequences corresponding respectively to the LTR region and to the  
25 gag gene for the retroviral genomic structure, in particular a nucleic fragment consisting of or comprising the sequence SEQ ID NO: 12.

The invention also relates to a nucleic material of the subgenomic retroviral type, consisting  
30 of a nucleotide sequence identical to SEQ ID NO: 11, with a deletion as exemplified by the clones cl.PH74 (SEQ ID NO: 7), cl.PH7 (SEQ ID NO: 8) and cl.Pi5T (SEQ ID NO: 9), this deletion resulting or otherwise from a splicing strategy.

35 The above-defined nucleic material comprises at least one functional nucleotide sequence encoding at least one retroviral protein, and/or at least one regulatory nucleotide sequence.

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Next, the invention relates to any nucleotide fragment of at least 100 bases, comprising a nucleotide sequence chosen from the group comprising:

5 a) all the nucleotide sequences, partial and complete, of a nucleic material as defined above

b) all the nucleotide sequences, partial and complete, of a clone chosen from the group including the clones:

- 10 - cl.6A2 (SEQ ID NO: 1)
- cl.6A1 (SEQ ID NO: 2)
- cl.7A16 (SEQ ID NO: 3)
- cl.Pi22 (SEQ ID NO: 4)
- cl.24.4 (SEQ ID NO: 5)
- cl.C4C5 (SEQ ID NO: 6)
- 15 - cl.PH74 (SEQ ID NO: 7)
- cl.PH7 (SEQ ID NO: 8)
- cl.Pi5T (SEQ ID NO: 9)
- cl.44.4 (SEQ ID NO: 10)
- HERV-W (SEQ ID NO: 11)
- 20 - cl.6A5 (SEQ ID NO: 12)
- cl.7A20 (SEQ ID NO: 13)
- cl.7A21 (SEQ ID NO: 14)
- LTR (SEQ ID NO: 15)

25 c) the sequences which are respectively complementary to the sequences according to a) and b)

d) the sequences which are respectively equivalent to the sequences according to a) to c), in particular the nucleotide sequences exhibiting, for any sequence of 100 contiguous monomers, at least 50%, and  
30 preferably at least 70%, or even better at least 80%, for example at least 90% homology with the sequences a) to c).

The invention also relates to any nucleic probe for the detection of a nucleic material, inserted or  
35 otherwise into a nucleic acid, characterized in that it is capable of hybridizing specifically with a nucleic material, as defined above.

Such a probe comprises a marker or otherwise.

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The invention also relates to a nucleic primer for the amplification by polymerization of an RNA or of a DNA, characterized in that it comprises a nucleotide sequence capable of hybridizing specifically with a nucleic material or a nucleic fragment, as defined above.

By way of example, a nucleic probe or nucleic primer according to the invention is characterized in that it consists of a nucleotide sequence chosen from the group including SEQ ID NOS: 16 to 28.

The invention also relates to any RNA or DNA, and in particular a replication vector, comprising a nucleotide fragment, as defined above.

The invention also relates to any peptide encoded by any open reading frame belonging to a nucleotide fragment, as defined above, in particular polypeptide, for example oligopeptide forming an antigenic determinant recognized by sera from patients affected by an autoimmune disease, or a pathology which is associated with it, or from patients having a pathological pregnancy or an unsuccessful pregnancy.

By way of example, this polypeptide is encoded by a nucleotide fragment comprising an open reading frame encoding one or more retroviral ENV proteins.

Finally, the invention relates to:

- the use of a nucleic material, or of a nucleotide fragment, or of a peptide defined above, as previously defined, as molecular marker for an autoimmune disease or for a pathology which is associated with it, for pathological pregnancy or unsuccessful pregnancy;

- the use of a nucleic material, or of a nucleotide fragment, as defined above, as chromosomal marker for susceptibility to an autoimmune disease or for a pathology which is associated with it, or for a risk of a pathological pregnancy or of an unsuccessful pregnancy;

- the use of a nucleic material, or of a nucleotide fragment, as defined above, as proximity

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marker for a gene for susceptibility to an autoimmune disease or to a pathology which is associated with it, or to a risk of a pathological pregnancy or of an unsuccessful pregnancy.

5           The invention also relates to a method for the molecular labeling of an autoimmune disease or of a pathology which is associated with it, of pathological pregnancy or of unsuccessful pregnancy, characterized in that any nucleotide fragment, as defined above,  
10 either in RNA form or in DNA form, is identified and/or quantified in any biological body material, in particular body fluid.

By way of example, according to such a method, cells expressing a nucleotide fragment, as defined  
15 above, are detected in said biological body material.

The invention relates to a diagnostic and/or therapeutic application of a nucleic material, of a nucleotide fragment or of a peptide defined above, and as such, another subject of the invention is a  
20 diagnostic composition or a therapeutic composition comprising said material, said fragment or said peptide.

Before detailing the invention, various terms used in the description and the claims are now defined:

25           - human virus is understood to mean a virus capable of infecting or of being harbored by a human being,

          - taking into account all the natural or induced variations and/or recombinations which may be  
30 encountered in the practical implementation of the present invention, the subjects thereof, defined above and in the claims, have been expressed comprising the equivalents or derivatives of the different biological materials defined below, in particular the homologous  
35 nucleotide or peptide sequences,

          - the variant of a virus or of a pathogenic and/or infective agent according to the invention comprises at least one antigen recognized by at least one antibody directed against at least one corres-

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ponding antigen of said virus and/or of said pathogenic and/or infective agent, and/or a genome of which any part is detected by at least one hybridization probe, and/or at least one nucleotide amplification primer specific for said virus and/or pathogenic and/or infective agent, in particular a genome belonging to the HERV-W family, under determined hybridization conditions well known to persons skilled in the art,

- according to the invention, a nucleotide fragment or an oligonucleotide or a polynucleotide is a stretch of monomers, or a biopolymer, characterized by the sequence, informational or otherwise, of the natural nucleic acids, capable of hybridizing with any other nucleotide fragment under predetermined conditions, it being possible for the stretch to contain monomers of different chemical structures and to be obtained from a natural nucleic acid molecule and/or by genetic recombination and/or by chemical synthesis; a nucleotide fragment may be identical to a genomic fragment of an element of the HERV-W family considered by the present invention, in particular a gene for the latter, for example pol or env in the case of said element;

- thus, a monomer may be a natural nucleotide of a nucleic acid, whose constituent elements are a sugar, a phosphate group and a nitrogen base; in RNA, the sugar is ribose, in DNA, the sugar is 2-deoxyribose; depending on whether DNA or RNA is involved, the nitrogen base is chosen from adenine, guanine, uracil, cytosine, thymine; or the nucleotide may be modified in at least one of the three constituent elements; by way of example, the modification may take place at the level of the bases, generating modified bases such as inosine, 5-methyl-deoxycytidine, deoxyuridine, 5-(dimethylamino)deoxyuridine, 2,6-diaminopurine, 5-bromodeoxyuridine and any other modified base promoting hybridization; at the level of the sugar, the modification may consist in the replacement of at least one deoxyribose with a

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polyamide, and at the level of the phosphate group, the modification may consist in its replacement with esters, in particular chosen from diphosphate, alkyl and arylphosphonate and phosphorothioate esters,

5                   - "functional" is understood to mean the characteristic according to which a nucleotide sequence, a nucleic material or a nucleotide fragment comprises an "an informational sequence",

10                   - "informational sequence" is understood to mean any ordered sequence of monomers whose chemical nature and the order in a reference direction, constitute or otherwise a functional information of the same quality as that of the natural nucleic acids, for example a reading frame encoding a protein, a  
15                   regulatory sequence, a splicing site or a recombination site,

20                   - hybridization is understood to mean the process during which, under appropriate operating, in particular, stringency, conditions, two nucleotide fragments, having sufficiently complementary sequences, pair to form a complex, in particular double or triple, structure, preferably in the form of a helix,

25                   - a probe comprises a nucleotide fragment synthesized in particular by the chemical or polymerization route, or obtained by enzymatic digestion or cleavage of a longer nucleotide fragment, comprising at least six monomers, advantageously from 10 to 100 monomers, preferably 10 to 30 monomers, and possessing a hybridization specificity under determined  
30                   conditions; preferably, a probe possessing less than 10 monomers is not used alone, but is used in the presence of other probes equally short in size or otherwise; under certain specific conditions, it may be useful to use probes larger than 100 monomers in size;  
35                   a probe may in particular be used for diagnostic purposes and it will include for example capture and/or detection probes,

                  - the capture probe may be immobilized on a solid support by any appropriate means, that is to say

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directly or indirectly, for example by covalence or by passive adsorption,

- the detection probe may be labeled by means of a marker chosen in particular from radioactive isotopes, enzymes particularly chosen from peroxidase and alkaline phosphatase and those capable of hydrolyzing a chromogenic, fluorogenic or luminescent substrate, chromophoric chemical compounds, chromogenic, fluorogenic or luminescent compounds, nucleotide base analogs, and biotin,

- the probes used for diagnostic purposes of the invention may be used in all the hybridization techniques known to persons skilled in the art, and in particular the techniques termed "DOT-BLOT", "SOUTHERN BLOT", "NORTHERN BLOT" which is a technique identical to the "SOUTHERN BLOT" technique but which uses RNA as target, the SANDWICH technique; advantageously, the SANDWICH technique is used in the present invention, comprising a specific capture probe and/or a specific detection probe, it being understood that the capture probe and the detection probe must have a nucleotide sequence which is at least partially different,

- any probe according to the present invention may hybridize in vivo or in vitro with RNA and/or with DNA, to block the phenomena of replication, in particular translation and/or transcription, and/or to degrade said DNA and/or RNA,

- a primer is a probe comprising at least six monomers, and advantageously from 10 to 30 monomers, possessing a hybridization specificity under determined conditions, for the initiation of an enzymatic polymerization, for example in an amplification technique such as PCR (Polymerase Chain Reaction), in an extension method such as sequencing, in a reverse transcription method and the like,

- two nucleotide or peptide sequences are said to be equivalent or derived from each other, or relative to a reference sequence, if functionally the corresponding biopolymers may play substantially the

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same role, without being identical, in relation to the application or use considered, or in the technique in which they are used; in particular equivalent are two sequences obtained because of the natural variability within the same individual, or the natural diversity from one individual to another within the same species, in particular spontaneous mutation of the species from which they were identified, or induced mutation, as well as two homologous sequences, the homology being defined below,

- "variability" is understood to mean any modification, spontaneous or induced, of a sequence, in particular by substitution, and/or insertion, and/or deletion of nucleotides and/or of nucleotide fragments, and/or extension and/or shortening of the sequence at at least one of the ends; an unnatural variability may result from the genetic engineering techniques used, for example from the choice of the synthetic primers, degenerate or otherwise, selected for amplifying a nucleic acid; this variability may result in modifications of any starting sequence, considered as reference, and which may be expressed by a degree of homology relative to said reference sequence,

- homology characterizes the degree of identity of two nucleotide or peptide fragments compared; it is measured by the percentage identity which is in particular determined by direct comparison of nucleotide or peptide sequences, relative to reference nucleotide or peptide sequences,

- this percentage identity was specifically determined for the nucleotide fragments, in particular clones within the present invention, and obtained from the same individual; by way of nonlimiting example, the lowest percentage identity observed between the different clones from the same individual (cf SEQ ID NOs: 13 and 14) is at least 90% and the lowest percentage identity observed between the different clones of two individuals is at least 80%,

- any nucleotide fragment is said to be equivalent to or derived from a reference fragment if it exhibits a nucleotide sequence equivalent to the sequence of the reference fragment; according to the above definition, particularly equivalent to a reference nucleotide fragment are:

(a) any fragment capable of at least partially hybridizing with the complement of the reference fragment,

(b) any fragment whose alignment with the reference fragment leads to identical contiguous bases being identified in a larger number than with any other fragment obtained from another taxonomic group,

(c) any fragment resulting or capable of resulting from the natural variability within the same individual, and from the natural diversity from one individual to another within the same species, from which it is obtained,

(d) any fragment capable of resulting from genetic engineering techniques applied to the reference fragment,

(e) any fragment, containing at least eight contiguous nucleotides, encoding a peptide homologous or identical to the peptide encoded by the reference fragment,

(f) any fragment different from the reference fragment by insertion, deletion, substitution of at least one monomer, extension, or shortening at at least one of its ends; for example, any fragment corresponding to the reference fragment, flanked at at least one of its ends by a nucleotide sequence not encoding a polypeptide,

- partial or complete nucleotide sequence of a reference nucleic material is also understood to mean any sequence associated by co-encapsulation, or by coexpression, or recombined with said reference nucleic material,

- polypeptide is understood to mean in particular any peptide of at least two amino acids, in

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particular oligopeptide or a protein, extracted, separated or substantially isolated or synthesized, through the intervention of human hands, in particular those obtained by chemical synthesis, or by expression  
5 in a recombinant organism,

- polypeptide partially encoded by a nucleotide fragment is understood to mean a polypeptide having at least three amino acids encoded by at least nine contiguous monomers contained in said nucleotide  
10 fragment,

- an amino acid is said to be analogous to another amino acid when their respective physico-chemical characteristics, such as polarity, hydrophobicity and/or basicity, and/or acidity, and/or  
15 neutrality, are substantially the same; thus, a leucine is analogous to an isoleucine,

- any polypeptide is said to be equivalent to or derived from a reference polypeptide if the compared polypeptides have substantially the same properties, and in particular the same antigenic, immunological, enzymological and/or molecular recognition properties; particularly equivalent to a reference polypeptide is:  
20

(a) any polypeptide possessing a sequence in which at least one amino acid has been substituted with an analogous amino acid;  
25

(b) any polypeptide having an equivalent peptide sequence obtained by natural or induced variation of said reference polypeptide, and/or of the nucleotide fragment encoding said polypeptide,

30 (c) a mimotope of said reference polypeptide,

(d) any polypeptide in whose sequence one or more amino acids of the L series are replaced by an amino acid of the D series, and vice versa,

(e) any polypeptide into whose sequence a  
35 modification of the side chains of the amino acids has been introduced, such as for example an acetylation of the amine functions, a carboxylation of the thiol functions, an esterification of the carboxyl functions,

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(f) any polypeptide in whose sequence one or more peptide bonds have been modified, such as for example the carba, retro, inverse, retro-inverse, reduced and methyleneoxy bonds,

5 (g) any polypeptide of which at least one antigen is recognized by an antibody directed against a reference polypeptide,

- the percentage identity characterizing the homology between two compared peptide fragments is, according to the present invention, at least 80% and preferably at least 90%.

The expressions relating to order which are used in the present description and the claims, such as "first nucleotide sequence" are not selected to express a particular order, but to define the invention more clearly.

Detection of a substance or agent is understood to mean hereinafter both an identification and a quantification, or a separation or isolation of said substance or of said agent.

*Brief Description of the Drawings*  
The invention will be understood more clearly upon reading the detailed description which follows, made with reference to the appended figures in which:

- Figure 1 represents, on the one hand, the organization of the endogenous retroviral material discovered according to the present invention, in the form of a putative genomic mRNA, and, on the other hand, the location of the clones used according to the present invention, relative to this organization; the scales for length are expressed in Kb; the flanking regions (5' UTR and 3' UTR) are indicated in hatched boxes; the regions repeated in these two flanking regions are indicated by black arrows; the regions corresponding to the gag, pol and env genes are indicated in black, white and gray respectively; the position of the Ppol-MSRV probe is indicated;

*Subca* - Figure 2 represents a possibility of genetic organization (DNA), illustrated by the clone RG083M05, and a splicing strategy linking to this sequence, the

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experimental clones (mRNA); this figure also shows the splicing sites observed with reference to the retroviral organization; additionally indicated in this figure are:

5 the location of the probes used (Pgag-LB19, Ppro-E, Ppol-MSRV and Penv-C15);

the splice donor sites (DS1 and DS2) and acceptor sites (AS1 to AS3);

CG clone 10 the sequences obtained from the clone RG083M05, in the lower-case boxes, and the sequences derived from experimental placental clones (mRNA), in the upper-case boxes;

the putative ORFs (ORF1, ORF2 and ORF3); and an insert of 2 Kb present in DNA form but not 15 detected in RNA form, represented in the form of vertical hatches.

The other conventions used in this figure are the same as those for Figure 1.

- Figure 3 gives a representation of genomic 20 (DNA) clones corresponding to the isolated cDNA clones; indicated in this figure are:

the percentage similarity with respect to the reconstructed genomic RNA (Recons RNA);

the presence of repeat sequences at each end of 25 these genomes (repeats); and

the presence and the size of the open reading frames (ORFs).

- Figure 4 represents a phylogenetic analysis identifying the HERV-W family.

30 - Figure 5 represents the alignment of the 5' and 3' flanking regions of the clone RG083M05 with the terminal 5' and/or 3' regions of some placental clones; the CAAC tandem flanking the 3' and 5' LTRs is doubly underlined under the DNA sequences, the consensus LTR 35 sequence of 783 bp (base pairs) is indicated under the alignment; the BPT upstream of the 5' end of LTR and the PBS downstream of the 3' end of LTR are indicated; the U3R and U5 regions are indicated; the sites corresponding to the binding of the transcription

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Sub  
OCB



factor are underlined and numbered from 1 to 6; the region -73 to 284 corresponds to the sequence evaluated in "CAT assay"; \* corresponds to putative sites for "capping"; [polyA] indicates the polyadenylation signal.

- Figure 6 represents a putative sequence of a HERV-W envelope polypeptide (ORF1) obtained from 3 different placental cDNA clones; the leader peptide (L), the surface protein (SU) and the transmembrane protein (TM) are indicated by arrows; the hydrophobic fusion peptide and the transmembrane carboxy region are underlined by a single line and a double line, respectively; the immunosuppression region is indicated in italics; the potential glycosylation sites are indicated by dots; the divergent amino acids are indicated on the bottom line; Figure 6 also presents the open reading frames corresponding to ORF2 and ORF3 as described in Figure 2, and more particularly their homologies with the retroviral regulatory genes.

*Detailed description of preferred embodiments*  
The nucleic material previously presented explicitly was discovered and characterized at the end of the experimental protocol described below, it being understood that this protocol cannot limit the scope of the present invention and of the accompanying claims.

#### Example 1

##### Isolation and sequencing of overlapping cDNA fragments

The information relating to the organization of HERV-W were obtained by testing a placental cDNA library (Clontech cat#HL5014a) with the probes Ppol-MSRV (SEQ ID NO: 29) and Penv-C15 (SEQ ID NO: 31) (cf Example 8), and then performing a "gene walking" technique with the aid of the new sequences obtained. The experiments were carried out with reference to the recommendations of the supplier of the library. PCR amplifications on DNA were also exploited in order to understand this organization.

A number of clones were selected and sequenced, see Figure 1:

C3  
C4

Sub  
C4

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HP2  
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- clone cl.6A2 (SEQ ID NO: 1): untranslated 5' region of HERV-W and part of gag

- clone cl.6A1 (SEQ ID NO: 2): gag and part of pol

5 - clone cl.7A16 (SEQ ID NO: 3): 3' region of pol

- clone cl.Pi22 (SEQ ID NO: 4): 3' region of pol and beginning of env

10 - clone cl.24.4 (SEQ ID NO: 5): spliced RNA comprising part of the untranslated 5' region of HERV-W, the end of pol and the 5' region of env

- clone cl.C4C5. (SEQ ID NO: 6): end of env and untranslated 3' region of HERV-W

15 - clone cl.PH74 (SEQ ID NO: 7): subgenomic RNA: untranslated 5' region of HERV-W, end of pol, env and untranslated 3' region of HERV-W

- clone cl.PH7 (SEQ ID NO: 8): multispliced RNA: untranslated 5' region of HERV-W, end of env and untranslated 3' region of HERV-W.

20 - clone cl.Pi5T (SEQ ID NO: 9): partial pol gene and U3-R region

- clone cl.44.4 (SEQ ID NO: 10): R-U5 region, gag gene and partial pol gene.

25 With the aid of these clones, by carrying out sequence alignments, a model of complete sequence of HERV-W was produced. The spliced RNAs were identified as well as the potential splice donor and acceptor sites. This set of information is shown in Figure 2. Through a study of similarity with existing  
30 retroviruses, the LTR, gag, pol and env entities were defined.

The putative genetic organization of HERV-W in RNA form is the following (SEQ ID NO: 11):

gene 1..7582

35 location of the clones on the reconstructed genomic RNA sequence

cl.6A2 (1321 bp) 1-1325;

cl.PH74 (535+2229= 2764 bp) 72-606 and 5353-7582;

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cl.24.4 (491+1457= 1948 bp); 115-606 and  
 5353-6810;  
 cl.44.4 (2372 bp) 115-2496;  
 cl.PH7 (369+297= 666 bp) 237-606 and 7017-  
 5 7313;  
 cl.6A1 (2938 bp) 586-3559.;  
 cl.Pi5T (2785+566= 3351 bp) 2747-5557 and  
 7017-7582;  
 cl.7A16 (1422 bp) 2908-4337;  
 10 cl.Pi22 (317+1689= 2006 bp) 3957-4273 and  
 4476-6168;  
 cl.C4C5 (1116 bp) 6467-7582  
 5' LTR 1..120  
 /note="R of 5' LTR (5' end uncertain"  
 15 121..575  
 /note="U5 of 5' LTR"  
 various 579..596  
 /note="PBS primer binding site for tRNA-W"  
 various 606  
 20 *Sub B2* /note="splice junction (splice donor site  
 ATCCAAAGTG-CTGAGTAATA and splice acceptor  
 site CTTTTTCAG-ATGGGAAACG clone RG083M05,  
 GenBank accession AC000064)"  
 various 5353  
 25 /note="splice acceptor site for ORF1 (env)"  
 various 5560  
 /note="splice donor site"  
 ORF 5581..7194  
 /note="ORF1 env 538 AA"  
 30 /product=="envelope"  
 various 7017  
 /note="splice acceptor site for ORF2 and  
 ORF3"  
 ORF 7039..7194  
 35 /note="ORF2 52 AA"  
 ORF 7112..7255  
 /note="ORF3 48 AA"  
 various 7244..7254  
 /note="PPT polypurine tract"

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3'LTR 7256..7582  
/note-="U3-R of 3' LTR (U3-R junction  
indeterminate)  
various 7563..7569  
5 polyadenylation signal

**Example 2:****Identification of genomic (DNA) clones corresponding to the isolated DNA clones**

A "blastn" interrogation of several databases,  
10 with the aid of the reconstructed genome, shows that a  
large quantity of related sequences exist in the human  
genome. About 400 sequences were identified in GenBank  
and more than 200 sequences in the EST library, and the  
majority as antisense. The 4 sequences most significant  
15 in size and in similarity, illustrated in Figure 3, are  
the following genomic (DNA) clones:

the human clone RG083M05 (gb AC000064) whose  
chromosomal location is 7q21-7q22,

the human clone BAC378 (gb U85196, gb AE000660)  
20 corresponding to the alpha delta locus of the T cell  
receptor, located in 14q11-12,

the human cosmid Q11M15 (gb AF045450) corres-  
ponding to the 21q22.3 region of chromosome 21,

the cosmid U134E6 (embl Z83850) on chromosome  
25 Xq22.

The location of the aligned regions for each of  
the clones is indicated and the affiliation to a  
chromosome is indicated in square brackets. The  
percentage similarity (without broad deletions) between  
30 the 4 sequences and the reconstructed genomic RNA is  
indicated, as well as the presence of repeat sequences  
at each end of the genome and the size of the largest  
reading frames (ORF). Repeat sequences are found at the  
ends of 3 of these clones. The reconstructed sequence  
35 is integrally contained inside the clone RG083M05  
(9.6 Kb) and exhibits a 96% similarity. However, the  
clone RG083M05 exhibits an insert of 2 Kb situated  
immediately downstream of the untranslated 5' region  
(5' UTR). This insert is also found in two other

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genomic clones which exhibit a deletion of 2.3 Kb immediately upstream of the untranslated 3' region (3' UTR). No clone contains the three functional reading frames (ORFs) gag, pol and env. The clone  
5 RG083M05 shows an ORF of 538 amino acids (AA) corresponding to a whole envelope. The cosmid Q11M15 contains two large contiguous ORFs of 413 AA (frame 0) and 305 AA (frame +1) corresponding to a truncated pol polyprotein.

10

**Example 3****Phylogenetic analysis**

A phylogenetic analysis was carried out at the level of the nucleic acids on 11 different subregions of the reconstructed genomic RNA, and at the protein  
15 level on 2 different subregions of env. All the trees obtained exhibit the same topology regardless of the region studied. This is illustrated in Figure 4 at the level of the nucleic acids in the most conserved LTR and pol regions between the sequences obtained and  
20 ERV-9 and RTLHV-H. The trees clearly show that the experimental sequences describe a new family distinct from ERV-9 and very distinct from RTLHV-H as underlined by the "bootstrap" analysis. These sequences are found on several chromosomes, in particular chromosomes 5, 7,  
25 14, 16, 21, 22 and X with a high apparent concentration of LTR on the X chromosome.

Comparison at the protein level between the most conserved regions of the retroviral env proteins shows that the HERV-W family is closer to the type D  
30 simian retroviruses and the avian reticuloendotheliosis retroviruses than the type C mammalian retroviruses.

This suggests a C/D chimeric genomic structure.

**Example 4****Identification of the LTR, PPT and PBS elements**

35 *subctb* The reconstructed sequence (RNA) is integrally contained inside the genomic clone RG083M05 (9.6 Kb) and exhibits a 96% similarity with two discontinuous regions of this clone which also contains repeat regions at each end. The alignment of the experimental

sequences corresponding to the 5' and 3' regions of the genomic RNA reconstructed with the DNA of the clone RG083M05 [5' (5-RG-28000-28872) and 3' (3-RG-37500-38314)] made it possible to deduce an LTR sequence and to identify elements characteristic of the retroviruses, in particular those involved in the reverse transcription, namely PBS downstream of the 5' LTR and the PPT upstream of the 3' LTR (cf Figure 5). It is observed that the U3 element is extremely short in comparison with that observed in the mammalian type C retroviruses, and is comparable in size to the U3 region generally described in the type D retroviruses and the avian retroviruses. The region corresponding to bases 2364 to 2720 of the clone cl.PH74 (SEQ ID NO: 7) was amplified by PCR and subcloned into the vector pCAT3 (Promega) in order to carry out the evaluation of the promoter activity. A significant activity was found in HeLa cells by the so-called "CAT assay" method showing the functionality of the promoter sequence of the LTR.

The PBS region is homologous to the PBS of the avian retroviruses.

#### Example 5

#### Genetic organization and regulation of expression

##### Organization in DNA form

PCR amplifications were carried out on whole HERV-W clones recovered on human genomic library (see Example 1 for the mode of production), using the following oligonucleotide pairs:

U5 4992 (SEQ ID NO: 16), GAG 4619 (SEQ ID NO: 17)  
GAG 4782 (SEQ ID NO: 18), POL 3167 (SEQ ID NO: 19)  
POL 3390 (SEQ ID NO: 20), POL 5144 (SEQ ID NO: 21)  
POL 5145 (SEQ ID NO: 22), U5 4991 (SEQ ID NO: 23).

The PCRs were carried out under the following conditions:

oligonucleotides at the concentration of 0.33 microMolar

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TAQ polymerase buffer Boehringer 1X  
0.5 unit of TAQ polymerase Boehringer  
mixture of dNTP at 0.25 mM each  
0.5 mg of human DNA

5 final volume 100 ml

PCR conditions (95°C, 5 min) × 1, (95°C, 30 sec  
+ 54°C, 30 sec + 72°C 3 min) × 35.

The PCR products were then deposited on 1%  
agarose gel to be analyzed after migration. The set of  
10 PCRs gives amplification fragments of the expected  
size, except for the LTR-4991--gag-4619 PCR which gives  
a fragment of size greater by about 2 Kb relative to  
the expected size (deduced from cDNAs from the  
placental library). The reconstruction of HERV-W in  
15 endogenous DNA form therefore represents an entity of  
about 10 Kb.

After cloning, sequencing and analysis of the  
PCR-4992 gag-4619, the presence of a region of  
insertion is observed between LTR and gag of  
20 SEQ ID NO: 12 (clone cl.6A5). This region does not  
correspond to an untranslated traditional region of a  
retrovirus: no  $\psi$  or PBS region.

The products of PCR pol-3390, pol-5144 were  
also cloned and two of the clones obtained were  
25 sequenced. The result of these sequences is given by  
the clones cl.7A20 (SEQ ID NO: 13) and cl.7A21  
(SEQ ID NO: 14). Comparison of these two nucleotide  
sequences gives a score of 90% homology for the  
relevant region, thus showing the variability of HERV-W  
30 in the same individual.

HERV-W in DNA form is proposed in Figure 2.

General organization: transcription process

The various cDNA clones having been obtained,  
results acquired in PCR on DNA, there is deduced:

35 - a DNA organization of 10 Kb possessing an  
insertion sequence of 2 Kb between LTR and gag.

The result of PCR on DNA showing the presence  
of an insert of 2 Kb between the LTR and gag regions  
suggests that the cDNAs isolated from the placenta are

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obtained from the expression of a genome of the RG083M05 type.

- an RNA organization of 8 Kb resulting from a transcription of 10 Kb followed by a splicing between LTR and gag making it possible to restore a continuity FR (Flanking Region) 5' gag, and thus giving an RNA of 8 Kb as identified in Northern blotting.

The probes gag (Pgag-LB19, SEQ ID NO: 30) and protease (Ppro-E, SEQ ID NO: 32) reveal an RNA having a size close to 8 Kb, the probe Penv-C15 (SEQ ID NO: 31) reveals, in addition, an RNA close to 3.1 Kb. Two probes defined in the untranslated 5' region, obtained by screening of the cDNA library reported above (probe P5'-gag-cl.6A2 derived from the clone cl.6A2 and probe P5'-env-cl.24.4 derived from the clone cl.24.4) reveal the preceding two RNAs and an RNA of about 1.3 Kb. This distribution of the RNAs is typical of complex retrovirus transcripts: a genomic RNA encoding gag-pro-pol, a subgenomic RNA encoding the envelope, and one or more multispliced RNAs potentially encoding regulatory genes.

The half-life of such an RNA (LTR-R-U5-Insertion-GAG-POL-ENV-U3-R-HERV-W) is probably very short, because no RNA of 10 Kb is detected in Northern blotting. By analyzing and comparing sequences, the potential splice donor sites (DS1 and DS2) and acceptor sites were defined and described in Figure 2.

#### Example 6

##### Transcription in healthy tissues

Various healthy human tissues were tested by the Northern-blot technique (Human Multiple Tissue Northern Blot, Clontech cat# 7760-1), with the aid of the probes Ppol-MSRV (SEQ ID NO: 29), Pgag-LB19 (SEQ ID NO: 30), Penv-C15 (SEQ ID NO: 31), Ppro-E (SEQ ID NO: 32), P5'-gag-cl.6A2 and P5'-env-cl.24.4, labeled as described in Example 1. The experiments were carried out following the recommendations of the manufacturers, and the autoradiographs were exposed for 5 days. Analysis of the results reveals transcription



products only in the placenta, and in none of the other human tissues tested (heart, brain, lungs, liver, skeletal muscle, kidney and pancreas).

Using an RNA Dot-Blot technique (Clontech: Human RNA Master Blot Cat# 7770-1), and using the experimental protocol recommended by the manufacturer, about forty other tissues, including fetal tissues, were tested: only the placenta gives a specific response after hybridization with the probes Pgag-LB19 (SEQ ID NO: 30) and Penv-C15 (SEQ ID NO: 31).

It is observed that a signal is observed in the kidney in RNA Dot-Blot, which is infirmed by the Northern-blot analysis.

#### **Example 7**

#### **Identification of an mRNA encoding an envelope and the means for detecting it specifically**

The screening of a placental cDNA library with the aid of a probe defined in the untranslated 5' region made it possible to isolate a cDNA defined by an untranslated 5' region (5' NTR), a splicing junction, a coding sequence, an untranslated 3' region (3' NTR) and a polyadenylated tail, cl.PH74 (SEQ ID NO: 7). This clone corresponds to a spliced RNA encoding an envelope. By comparing sequences between this cDNA and the endogenous HERV-W model proposed according to Figure 2, a splicing junction is identified on the mRNA, a splicing junction placing in continuity the 5' NTR region and the env gene, leading to the production of a spliced subgenomic RNA encoding the envelope gene. This information made it possible to define an oligonucleotide specific for this mRNA by choosing a location situated on the splicing site (Oligo 5307, according to SEQ ID NO: 24).

The identification of this joining region makes it possible to establish a method of discriminating between endogenous retroviral RNA and DNA, using, in a PCR, an oligonucleotide defined on this joining region, in particular an oligonucleotide chosen from the env gene (Oligo 4986, according to SEQ ID NO: 25).

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The PCRs were carried out under the following conditions:

oligonucleotides at the concentration of 0.33 microMolar

- 5           TAQ polymerase buffer Boehringer 1X  
          0.5 unit of TAQ polymerase Boehringer  
          mixture of dNTP at 0.25 mM each  
          0.5 mg of human DNA  
          final volume 100 ml

- 10           On 10 different DNAs tested, this type of PCR  
did not make it possible to obtain amplification  
products. On the other hand, on cDNA derived from  
placental RNA or from cells expressing HERV-W, this PCR  
gives an amplification product. This result therefore  
15 confirms the specifically RNA nature of this subgenomic  
fragment.

#### **Example 8**

**Identification of coding sequences contained in  
a specific mRNA**

- 20           The splicing strategy described in Example 5 is  
compatible with the presence of three reading frames  
ORF1 (SEQ ID NO: 33), ORF2 (SEQ ID NO: 34) and ORF3  
(SEQ ID NO: 35) (cf Figure 6).

- The screening of a placental cDNA library made  
25 it possible to isolate a cDNA (SEQ ID NO: 7, cl.PH74)  
defined by an untranslated 5' region (5' NTR), a  
splicing junction, a coding sequence, an untranslated  
3' region (3' NTR) and a polyadenylated tail. The  
coding sequence is 538 amino acids (SEQ ID NO: 33). The  
30 analyses carried out on databanks make it possible to  
identify characteristics of a complete retroviral  
envelope: initiation of translation of an envelope  
polyprotein, of a highly hydrophobic leader peptide of  
about 21 amino acids, of a surface protein SU, of a  
35 transmembrane protein TM. These two protein entities  
exhibit different potential glycosylation sites. An  
immunosuppressive region is identified within the TM  
protein.

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22 bp and 95 bp upstream of the splice acceptor site, two initiation codons were respectively found which were capable of directing the synthesis of 52 AA (ORF2, SEQ ID NO: 34) and of 48 AA (ORF3, SEQ ID NO: 35). ORF2 consists of part of the carboxy-terminal end of env and ORF3 corresponds to a different but overlapping translation.

No significant homology was found by "blast" interrogation. However, an LFASTA interrogation in a sub-databank limited to the Retroviridae, ORF2 and ORF3 showed a percentage identity of 35% with, respectively, Rex of the human and primate lymphotropic T virus, and with Tat of the simian immunodeficiency virus.

#### Example 9

##### Complexity of the HERV-W family

The number of copies present in the human genome of each of the sequences is evaluated by a Dot-Blot technique, with the aid of the probes P<sub>gag</sub>-LB19 (SEQ ID NO: 30), P<sub>pro</sub>-E (SEQ ID NO: 32) and P<sub>env</sub>-C15 (SEQ ID NO: 31).

Each of the probes is denatured and deposited on a Hybond N+ membrane in an amount of 2.5, 5, 10, 25, 50, 100 pg per deposit. 0.5 mg of human DNA are also deposited on the same membrane. The membranes are dried for 2 hours under vacuum at 80°C. The membranes are then hybridized with the deposited probe. The techniques for labeling the probes, for hybridization and for washing the membranes are the same as for the Southern blotting. After autoradiography of the membranes, levels of signal intensity which are proportional to the deposits on the membrane are observed. After cutting out the hybridization zones, scintillation counting is carried out. By comparison between the dilution series for the probe deposited on the membrane and the result obtained with the human DNA, it is possible to evaluate the number of copies per haploid genome of each of the regions covered by the probes:

- the number of endogenous gag is evaluated from 56 to 112 copies (76)

- the number of endogenous protease is evaluated from 166 to 334 copies (260)

5       - the number of endogenous env is evaluated at less than 52 copies (13).

10       The screening of  $10^6$  clones of a human placental DNA library (Clontech cat# H15014b) made it possible to count 144 clones recognized by the probe Pgag-LB19, and 64 clones recognized by the probe Penv-C15. 13 clones hybridized conjointly with the probes Penv-C15 and Pgag-LB19 were isolated, confirming the presence of several copies of a genome possessing both gag and env, without consideration of  
15       functionality.

20       The nucleic material, the nucleotide sequences and the peptides or proteins which may be expressed by said materials and sequences may be used to detect, predict, treat and monitor any autoimmune disease, and the pathologies which are associated with it, as well as in cases of pathological pregnancy or of unsuccessful pregnancy.

25       Indeed, the objective and experimental data make it possible to link retrovirus and autoimmune diseases and retrovirus and pregnancy disorders:

30       (1) common mechanisms are used in the retroviral pathologies and in autoimmune diseases (presence of autoantibodies, of immune complexes, cellular infiltration of certain tissues, neurological disorders).

35       (2) pathological disorders comparable to certain autoimmune diseases appear during infections with HIV and HTLV retroviruses (Sjögren syndrome, disseminated lupus erythematosus, rheumatoid arthritis and the like).

      (3) a reverse transcriptase activity was detected and retroviral-type particles were observed in the cell culture supernatants of patients suffering from multiple sclerosis (Perron et al., Res. Virol.

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1989; 140: 551-561/Lancet 1991; 337: 862-863/Res. Virol. 1992; 143: 337-350) or from rheumatoid arthritis.

(4) autoimmune or chronic inflammatory animal pathologies are linked to endogenous retroviruses; some of them are used as animal models of human diseases (insulin-dependent diabetes, disseminated lupus erythematosus).

(5) significant levels of endogenous anti-retrovirus antibodies have been described in the context of autoimmune, systemic or inflammatory diseases; other data of this nature were communicated by several authors at the IVth European meeting on endogenous retroviruses (Uppsala, October 1996). According to Venables (communiqués of the IVth European meeting on endogenous retroviruses, Uppsala, October 1996), a significantly high level of anti-HERV-H antibodies are found during pregnancy but also in the context of various autoimmune disorders such as Sjögren syndrome, disseminated lupus erythematosus or rheumatoid arthritis, without, however, any proof of its direct involvement being provided up until now.

The involvement of the retroviruses in the autoimmune phenomenon remains compatible with the multifactorial character of the autoimmune, systemic or inflammatory diseases which confront genetic, hormonal, environmental and infectious factors.

The particles observed in the cell culture supernatants from patients suffering from multiple sclerosis (Perron et al., Res. Virol. 1989; 140: 551-561/Lancet 1991; 337: 862-863/Res. Virol. 1992; 143: 337-350) or from rheumatoid arthritis (unpublished data) may result from the expression: (i) of an endogenous retrovirus competent for replication, (ii) of several defective endogenous retroviruses cooperating by a phenomenon of transcomplementation or (iii) of an exogenous retrovirus.

All these observations make it possible to use and consider the above-described biological material as

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marker for an autoimmune disease or for pregnancy disorders.

In particular, the following labeling techniques are considered:

- 5           - screening of the human genome with high-stringency hybridization probes derived from the nucleic material described above,
- direct amplification of genomic DNA by PCR, using primers specific for the region considered
- 10          - analysis of the flanking regions of foreign cellular genes.

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